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Original scientific paper

CLONAL FIDELITY OF CHRYSANTHEMUM REGENERATED FROM LONG TERM CULTURES

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Morphological characteristics of flowers of long term regenerated chrysanthemum, *cv*. "White Spider", after ten years of micropropagation are investigated. Shoot cultures are established and maintained more than ten years by stem segment culture on MS medium supplemented with BAP and NAA (1.0, 0.1 mgL^{-1}, respectively). Rooting of shoots (100 %) has done on MS medium without hormones and it was very successful after ten years, as well as, after two or eight years of micropropagation. Acclimation of rooted chrysanthemum plantlets at greenhouse conditions was excellent and after appropriate photoperiod "*in vitro*" plants flowered 90.3 % and have the same flower color, shape and size as mother plants. Flower color changes of "*in vitro*" plants are observed during another flowering cycle one year after acclimatization. Observed variations of chrysanthemum flowers could be attributed to epigenetic factors.

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INTRODUCTION

Chrysanthemums (*Dendrantema* x *grandiflora* (Ramat.) Kitamura, *Asteraceae*), commonly called autumn queen are one of the three most important cut and pot plants grown in many parts of the world. It was bred in China around 2500 years ago. The garden chrysanthemum is hexaploid with 54 chromosomes (NAZEER and KHOSHOO, 1983) and has very strong self-incompatibility system (DREWLOW et al., 1973). Commercial cultivars are obtained by traditional breeding and more recently by genetic and molecular techniques (GUTTERSON et al., 1994, ZUKER et al., 1998).

Chrysanthemums are propagated vegetatively by root suckers or terminal cuttings. Clonally propagation by *in vitro* culture using shoot proliferation from different type of culture provides a useful method for obtain of genetically uniform plants. *In vitro* regeneration of chrysanthemums has been reported, for the first time about forty years ago and many others using different explants and nutritional media (HILL, 1968). There are numerous reports of adventitious shoot regeneration from various explants of chrysanthemum but in most cases shoots were produced from a callus intermediary (DA SILVA, 2004).

Plant regeneration by stem culture of different chrysanthemum cultivars has studied more that 10 years in our laboratory (RADOJEVIĆ et al., 1994, 2000, 2001; JEVREMOVIĆ et al., 2004). Main goal of this work is to compare some growth characteristics as well as flower characteristics of micropropagated plants after long term culture.

MATERIALS AND METHODS

Shoots of one flowered plant of cultivar "White Spider" from greenhouse "Kanjiza" are used for induction of morphogenesis *in vitro*. Sterilization of plant material, as well as, establishment of stem segment culture was as previously described (JEVREMOVIĆ et al., 1995,). All nutritional media are composed of MURASHIGE and SKOOG (1962) mineral solution, 3% sucrose, 0,7% agar and (in mg L^{-1}): inositol 100, vitamin B_1 30, nicotinic acid 10, tirozin 100 and adenine sulphate 80 (medium MS). Shoot cultures are established in 1996 and multiplied on MS + NAA + BAP (0.5 and 1.0 mg L^{-1}, respectively) by stem culture since today. Shoots (2 cm) are rooted on MS hormone free medium. All cultures were grown on 16 h day/8 h night photoperiod on light intensity 50 μmol m^{-2} s^{-1} at temperature 25°C ± 1°C. Height, number of roots and the length of the longest root are measured before potting. Rooted plantlets are potted in mixture of peat: perlite (3:1) in May each year and flowered during October and November. All data are analyzed using ANOVA and LSD (least significant differences) test for statistical analyses by STATGRAF and ORIGIN 7.0 computer program.

RESULTS AND DISCUSSION

Nodal and internodal segments of *cv*. "White Spider" are cultured on MS + NAA + BAP (0.5 and 1.0 mg L^{-1}, respectively) and shoots are induced (93.7 %) directly without callus phase, after four weeks. Shoots have multiplied in stem segment culture by axillary shoots on the same media until today. Shoot multiplication index is followed during nineteen subcultures (three years). Decrease of shoot multiplication index is observed and varies from 6.8 at start of culture to 1.8 after tree years. Plantlets from a range of chrysanthemum cultivars are produced by direct adventitious shoot regeneration and addition of BAP and NAA (1.0, 0.5 mgL^{-1}, respectively) to the culture medium was essential (JEVREMOVIĆ and RADOJEVIĆ, 2004; DA SILVA, 2004).

A method of producing shoots, ultimately from single cells and directly from explants tissue not callus, and may help retain clonal fidelity (BROERTJES and KEEN, 1980).

Table 1. Rooting of shoots cv. "White Spider" Chrysanthemum morifolium followed after two, eight ant ten years in stem segment culture

Rooting			
Year	Percent (%)	Roots	
		N^0	Length of longest root
1998.	97.1	8.6 ± 0.4 a *	66.0 ± 3.2 a
2004.	100	7.9 ± 0.2 a	158.9 ± 3.5 c
2006.	100	11.3 ± 0.5 b	83.4 ± 2.4 b

* Each value represent the mean ± standard error of at lest 50 plantlets. Different letters indicate significant differences ($P \leq 0.05$) between years using LSD test.

Rooting of shoots *cv*. "White Spider" was very successfully after ten years, as well as, after two years of micropropagation (Table 1.). Height of regenerated plants was similar after eight and ten years of micropropagation (Fig.1). Acclimation of rooted chrysanthemum plantlets at greenhouse conditions was excellent (97%). After appropriate photoperiod, 90.3 % plants flowered and have same flower color as mother plants. Some morphological changes of florets are observed (Plate 1.).

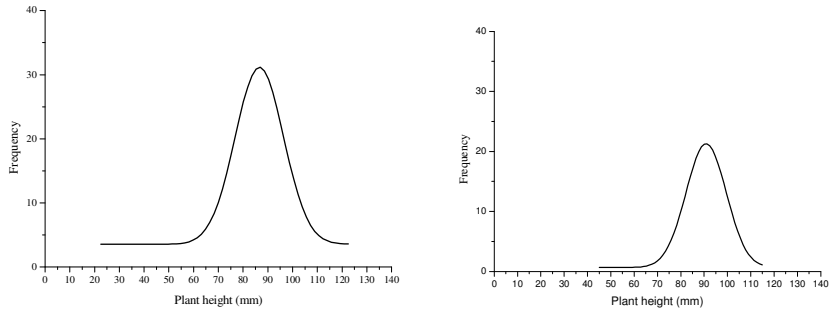

Fig.1. Height distribution of chrysanthemum plantlets *cv*. "White Spider" plantlets after eight (left) and ten years (right) of micropropagation

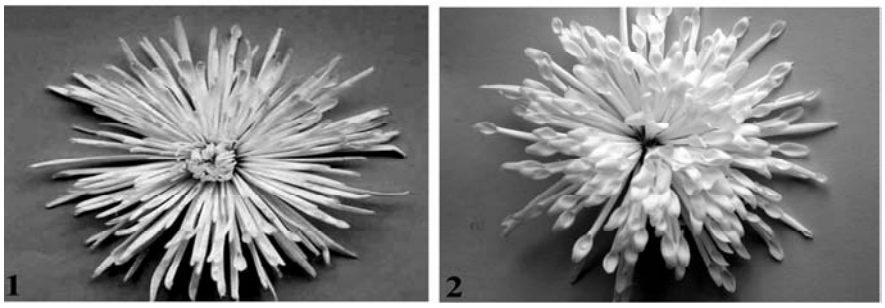

Plate 1. Flower of chrysanthemum *cv*. "White Spider" at unset of culture *in vitro* (left) and flower of same cultivar after eight years of micropropagation (right).

MALAURE et al., (1991) reported that shoots derived from florets of 16 cultivars of chrysanthemum showed more variation than plants regenerated from vegetative parts. Plants grown in a glasshouse derived from either nodal cultures harvested from leaf and stem explants had similar leaf size and shape, growth habit and flower color (KAUL et al., 1990).

SUTTER and LANGHANS (1981) reported that the plants derived from long-term cultures showed delayed flowering and the addition of GA_3 or IAA to the culture medium had no effect in restoring normal flowering.

Flower color changes of "*in vitro*" plants are observed in next flowering period, one year after acclimatization. Observed somaclonal variations of

chrysanthemum flowers could be attributing to epigenetic factors. The truly nature of observed variations can be detect by RAPD analysis. The RAPD analysis technique is proven to be very useful and rapid method for detection of changes produced during plant tissue culture (WOLFF and PETERS-VAN RIJN (1993), MARTIN et al., 2002).

CONCLUSION

Plant regeneration of *cv.* White Spider has been reported earlier by *in vitro* culture of axillary buds on media supplemented with 0.1-0.5 kinetine (DABIN and CHOISEG, 1983). There was no data about clonal fidelity, especially after long term culture of this cultivar.

Addition of NAA and BAP to the culture medium was essential for many regeneration protocols as well as for "White Spider". Clones of micropropagated chrysanthemum after 8 years of multiplication in culture *in vitro* indicate some morphological but not flower color changes. The truly nature and degree of changes at genetic level can be revealed only by more sophisticated methods like application of RAPD markers.

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KLONALNA NEPROMENLJIVOST HRIZANTEME POSLE DUGOTRAJNE MIKROPROPAGACIJE

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I z v o d

Praćene su morfološke karakteristike cvetova regeneranata cv. "White Spider" posle deset godina gajenja u kulturi *in vitro*. Izdanci su umnožavani u kulturi segmenata stabla preko aksilarnih pupoljaka na MS podlozi sa α-naftilsirćetnom kiselinom i benzilaminopurinom (0,5 odnosno 1,0 mgL^{-1}), . Praćeno je umnožavanje izdanaka u periodu od devetnaest pasaža (tri godine) kada je došlo do sukcesivnog smanjenja indeksa umnožavanja. Ožiljavanje izdanaka je praćeno na MS podlozi bez hormona i bilo je podjednako uspešno posle dve, kao i posle osam i deset godina mikropropagacije. Ožiljene biljke hrizantema su uspešno aklimatizovane na uslove staklenika (97 %). Nakon odgovarajućeg fotoperioda, 90,3 % biljaka je cvetalo i imale su indentičnu boju cveta matičnim biljkama hrizantema. Promene u boji cveta su uočene kod "*in vitro*" biljaka u sledećem ciklusu cvetanja, tj. godinu dana posle aklimatizacije. Ove promene bi se mogle pripisati epigenetskim faktorima.

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